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Prevalence and distribution of antimicrobial resistance in effluent wastewater from animal slaughter facilities: A systematic review

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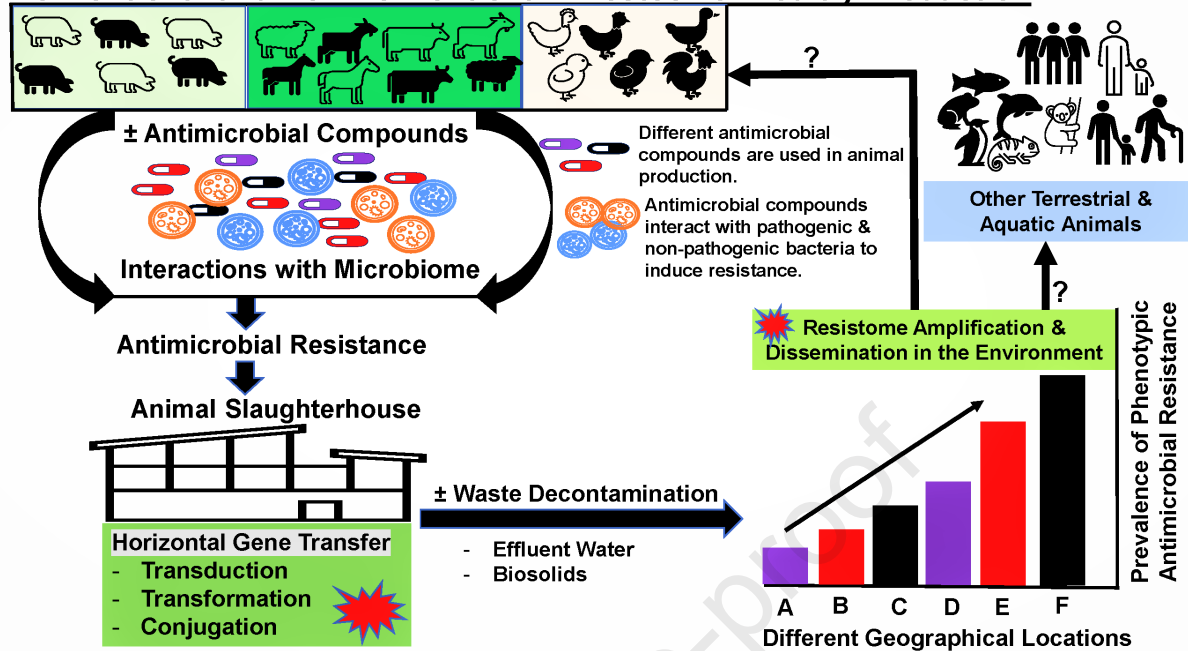
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Conventional and Non-Conventional Livestock or Poultry Production



**Prevalence and Distribution of Antimicrobial Resistance in Effluent
Wastewater from Animal Slaughter Facilities: A systematic Review**

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Running title: Antimicrobial resistance in wastewater from food animal
slaughter facilities: A global One Health problem

Abstract

The extensive use of antibiotics in food animal production and disposal of untreated
wastewater from food animal slaughter facilities may create a shift in microbiomes of
different ecosystems by generating reservoirs of antimicrobial resistance along the
human-animal-environmental interface. This epidemiological problem has been studied,
but its magnitude and impact on a global scale is poorly characterised.

A systematic review was done to determine global prevalence and distribution

patterns of antimicrobial resistance in effluent wastewater from animal slaughter facilities. Extracted data were stratified into rational groups for secondary analyses and presented as percentages. Culture and sensitivity testing was the predominant method; *Escherichia* spp., *Enterococcus* spp., and *Staphylococcus aureus* were the most targeted isolates. Variable incidences of resistance were detected against all major antimicrobial classes including reserved drugs such as ceftazidime, piperacillin, gentamicin, ciprofloxacin, and chloramphenicol; the median frequency and range in resistant Gram-negative isolates were: 11 (0-100), 62 (0-100), 8 (0-100), 14 (0-93) and 12 (0-62) respectively. Ciprofloxacin was the most tested drug with the highest incidences of resistance in livestock slaughterhouses in Iran (93%), Nigeria (50%) and China (20%), and poultry slaughterhouses in Germany (21-81%) and Spain (56%). Spatial global distribution patterns for antimicrobial resistance were associated with previously reported magnitude of antibiotic use in livestock or poultry farming and, the implicit existence of jurisdictional policies to regulate antibiotic use. These data indicate that anthropogenic activities in farming systems are a major contributor to the cause and dissemination of antimicrobial resistance into the environment via slaughterhouse effluents.

Keywords: Abattoir; Slaughterhouse; Antibiotic Resistance Genes; Microbiome, One Health; Livestock

1. Introduction

The discovery of major classes of antibiotics early in the 20th century was accompanied by optimism regarding the management of bacterial infections. In the 21st century however, antimicrobial resistance (AMR) is a growing crisis that threatens our ability to manage infectious conditions in humans and animals alike (World Health Organisation, 2021; Bengtsson and Greko, 2014). To face this challenge, the World Health Organisation has commissioned a series of action plans that include increasing awareness of the magnitude and impact of AMR through surveillance and research, education, training, and communication (WHO, 2017). The rapid development of AMR has risen mainly from the overuse and misuse of antibiotics in human and animal health, and in agricultural settings (English and Gaur, 2010; Manyi-Loh et al., 2018). Progressively, these practices have created antimicrobial selection pressure within microbial populations, and this has enhanced the acquisition and expression of AMR genes for bacteria to survive. Reservoirs of antibiotic residues, antibiotic resistant bacteria, or genes responsible for conferring resistance to antibiotics (ARG) are detectable in humans, companion animals, health clinics and hospitals, animal farm and aquaculture settings, soil, wildlife, and many different ecological systems (Marti et al., 2014; Huijbers et al., 2015; Holmes 2016; Burow and Kasbohrer, 2017). The danger associated with AMR is heightened by the potential to involve or transfer to deadly zoonotic bacteria. Of particular concern are a group of Gram-positive and Gram-negative bacteria identified as ESKAPE pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Enterobacter species*), because of the very high virulence and ability to acquire and spread resistance against multiple classes of commonly used antibiotics (Rice, 2008; Mulani et al., 2019). The ESKAPE group of pathogens are associated with most

nosocomial infections in hospitals (Rice, 2008), but many other highly pathogenic and zoonotic bacteria such as *Escherichia* spp., *Salmonella* spp. and *Campylobacter* spp. are also identifiable in undomesticated animals and environmental matrices along the entire One Health continuum (Mulani et al., 2019; Santaniello et al., 2020). To a large extent, because of recent advances in molecular biology techniques, mechanisms that underpin the phenotypic expression of AMR in most bacteria are known and this topic has been extensively reviewed by other scholars (McEwen and Collignon, 2018; Chen et al., 2020; de Abreu et al., 2021). For instance, established comprehensive antibiotic resistance gene databases are now being used to identify pathogenic bacteria and entire antimicrobial resistance profiles in different matrices (Founou et al., 2018; Chen et al., 2020; Abreu et al., 2021). Despite all these advances however, there has remained an unmet need to determine and fully characterise, all epidemiological factors contributing to AMR along the One Health continuum. The development and spread of AMR have remained a big problem because of the complex nature of interactions relating to antimicrobial use in humans, animals, and the environment. In addition, it is becoming increasingly clear how widespread active pharmaceutical ingredients, including antimicrobials are in global ecosystems (Wilkinson et al., 2022).

Relative to other epidemiological factors such as antimicrobial misuse in humans and animals in health care facilities and agriculture, the spread of AMR in the environment is classified as an important, moderate level risk to One Health (Huijbers et al., 2015; McEwen and Collignon, 2018). It is critically important therefore, to determine epidemiological drivers of environmental AMR. This is because of the potential for antibiotics to shift environmental microbiomes by selecting for antibiotic resistant phenotype and exert selection pressure to create reservoirs of environmental AMR (Ben et al., 2019). These issues are demonstrated by many examples, but a few specific cases

101 include the identification of extended-spectrum beta-lactamase producing
102 Enterobacteriaceae (Schmiege et al., 2021; Savin et al., 2022), methicillin-resistant
103 *Staphylococcus aureus* (Zhou et al., 2018; Back et al., 2020; Odetokun et al., 2022), and
104 vancomycin-resistant *Enterococcus* spp. (Biggel et al., 2021; Tzavaras et al., 2021), in
105 different environmental milieus across different geographical locations. It is also notable
106 that the dissemination of environmental AMR is mostly associated with many different
107 anthropogenic activities and one such activity is the processing and disposal of
108 wastewater from abattoirs or animal slaughterhouses. Abattoir and/or slaughterhouse
109 wastewater is likely to be a point of convergence and concentration of antibiotics,
110 bacteria, and ARG; this is likely to be exacerbated by the fact that all major classes of
111 antibiotics including beta-lactams, macrolides, quinolones, aminoglycosides,
112 tetracyclines and potentiated sulphonamides are used in livestock or poultry farms across
113 many different geographical jurisdictions (van Boeckel et al., 2015; ECDC/EFSA/EMA,
114 2017; EMA, 2019). To date, a lot of studies have focussed on characterising antibiotic
115 residues and AMR in non-pathogenic or pathogenic bacteria in livestock and poultry
116 products for human consumption (Murphy et al., 2018; Bager et al., 2019). A specific
117 critical appraisal of the global prevalence and distribution of AMR associated with
118 abattoir/slaughterhouse wastewater is lacking. In a broader context though, technologies
119 to remove ARGs from aquatic environments have been developed and evaluated (Li et
120 al., 2021), but the worldwide application of these technologies to remove ARGs from
121 animal slaughterhouse effluents is poorly understood. The primary objective of this this
122 study was to systematically review published, peer reviewed literature to determine
123 relative global prevalence and distribution patterns of AMR associated with abattoir
124 and/or slaughterhouse wastewater effluents.

125

2. Methods

2.1 Literature search strategy

This study was carried out according to the 2009 Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement (Moher et al., 2009). The specific aim was to undertake a systematic review of the published literature and determine the occurrence and distribution of AMR against the major classes of antimicrobial agents in slaughterhouse wastewater. Preliminary evaluations indicated that peer reviewed, primary sources of this type of literature are predominantly archived in Pubmed/Medline, Web of Science and Science Direct. These 3 databases were searched from dates of inception to October 2021. The utilised specific search terms were (Slaughterhouse OR Abattoir) (Effluent OR Wastewater OR Sludge) AND (Antibiotic resistance OR Antimicrobial resistance) in medical subject headings as well as keyword searches. The search strategy purposely excluded grey literature with an emphasis on peer reviewed, primary research articles. Publications not written in English without translations were also excluded. After the full search, all article titles, abstracts and accompanying reference lists were screened to remove irrelevant records to generate a comprehensive reference list. Duplicate records across the three databases were removed using Endnote Version 20.

2.2 Study inclusion and exclusion criteria, data extraction and quality assessment

To match the established research objective, the inclusion criteria focussed on wastewater destined for release in the environment or secondary treatment plants, and the phenotypic quantification of AMR against tested antibiotics with or without the determination of genetic mechanisms of resistance. Studies on AMR associated with other microenvironments in animal slaughter facilities such as meat products, meat processing surfaces, abattoir floors, equipment, or personnel were not in the scope of this review and these data were not extracted. Studies in which AMR was not quantified or defined relative to specific bacterial species and specific antibiotics were

also excluded. Research articles deemed to be relevant to the topic were further assessed and individually qualified for data extraction by all authors. All relevant articles were accessible, and data were extracted from text, tables, figures, and supplemental files of the respective publications. Extracted information included: number and specific location of animal slaughterhouses, animal species in the slaughter facility, number of wastewater samples, wastewater treatment methods or disposal procedures, bacterial species and number of isolates tested for phenotypic expression of AMR, the number and frequency (percentage) of resistant isolates against each tested antibiotic and, the type as well as frequency of identified genetic markers for AMR. The inclusion criteria and the extracted information were used to design a ten-point scoring system for the qualitative evaluation of individual publications.

2.3 Analysis and presentation of extracted data

Extracted data were summarized and presented as qualitative descriptions. Quantitatively, the frequency of the phenotypic expression of AMR, and the frequency of genetic determinants of AMR in tested isolates were expressed as percentages. For qualitative appraisal, percentages of phenotypic and genetic expression of AMR were stratified into rational groups including nation in which the study was conducted, target animal species for the specific abattoir or slaughterhouse, classification of the bacterial species in tested isolates and, the type of antimicrobial drug tested. Due to marked heterogeneity in experimental conditions and generated data across all studies presented here, no additional meta-analyses and statistical evaluations were done.

3. Results

3.1 Quantity and qualitative features of evaluated studies

A total of 968 publications were identified from database searches but 71 of these were duplicate records and 196 were encyclopaedia, review articles and book chapters that were removed from any

further evaluations (Fig. 1). The remaining 703 primary research articles were then evaluated against the inclusion criteria and 37 records met the outlined inclusion qualitative assessment with relevant meta-data, but only 27 articles were eligible for secondary data analyses (Fig. 1, Fig. 3, Supplemental Table 1 and references contained therein). Studies that met the inclusion criteria represented a very limited number of countries in Western Europe, Africa, South America, Asia, and Australia (Fig. 2). It was not possible to determine the precise number of animal slaughterhouses studied in each of the countries with multiple records, and this was because of the lack of specificity in the recorded geographical locations. Out of a cumulative record of 133 animal slaughter facilities, 51 (38%) represented mixed or unidentified animal species, 42 (32%) represented poultry, 27 (20%) represented ruminants, and pigs were represented by 13 (10%). The global distribution of the studied animal slaughter facilities is summarised in fig. 2.

There was marked variation in assessed qualitative features of all included studies and the average percentage score for all quality parameters was 61 ± 15 . All included studies met the strict criteria of having performed tests for the phenotypic expression of AMR in animal slaughterhouse wastewater, but only 46% of these studies attempted to elucidate some of the genetic mechanisms of AMR (Fig. 3). In most assessed studies (31/37), the number of wastewater samples used was defined, but only 5/37 (14%) attempted to rationalise sample size or outline a sampling strategy. Slaughterhouse wastewater disposal processes were generally very poorly defined and only 12/37 (32%) of included studies from Brazil, France, Germany, Portugal, Spain, and Taiwan, described elaborate processing of wastewater prior to release in the environment. There was a lot of heterogeneity in the outlined wastewater treatment processes; these included the use of lagoons, anaerobic and facultative stabilising ponds, aeration of activated sludge with clarification and dichlorination, and membrane bioreactors with immersed ultrafiltration (Supplemental Table 1). All other qualitative measures assessed herein are outlined in Fig. 3.

3.2 Gram-positives bacteria and associated phenotypic AMR patterns

Testing for phenotypic expression of AMR was done against specific culturable Gram-positive and Gram-negative bacterial isolates. For Gram-positive isolates, most characterised isolates were *Enterococcus* spp. and Methicillin-resistant *Staphylococcus aureus* (MRSA) representing 48% of all tested isolates (Fig. 4). Among the MRSA and *Enterococcus* spp, screening for resistance was done against many different antimicrobials of the beta-lactam class including penicillins and potentiated penicillins, first to fifth generation cephalosporins, and carbapenems (Fig. 5A). However, this was only done in five countries including Germany, Portugal and Malaysia that represented poultry; Nigeria and Turkey that represented livestock slaughterhouses. The highest frequency of resistance was observed against penicillins and, first or second generation cephalosporins in all represented countries except Portugal and Turkey. For carbapenems and extended spectrum cephalosporins, a relatively high (100%) frequency of resistance against ceftazidime was observed in Nigeria, but relatively low incidences of resistance were observed against ceftriaxone in Nigeria (27%), meropenem in Turkey (12%) and Ceftaroline in Germany (8%).

Representative Gram-positive bacteria were also screened for phenotypic resistance against several non-beta-lactam antimicrobial agents (Fig. 5B). In three countries (Germany, Portugal and Malaysia) representing poultry slaughterhouses, and two countries (Germany and Taiwan) representing pig slaughterhouses, the highest cumulative frequency of resistance was against erythromycin, tetracycline and clindamycin (Fig. 5B). Generally, there was a relatively low frequency of resistance against moxifloxacin, gentamicin, chloramphenicol, quinupristin-dalfopristin, rifampin, nitrofurantoin, vancomycin, kanamycin, ciprofloxacin and norfloxacin. The exceptions to this were in wastewater from a pig slaughterhouse in Taiwan with 100% resistance against gentamicin and 45% resistance against chloramphenicol, and wastewater from poultry slaughterhouses in Portugal with a 56% incidence of resistance against kanamycin in vancomycin-resistant *Enterococcus* spp. (Fig. 5B).

3.2 Gram-negative bacteria and associated phenotypic AMR patterns

Gram-negative isolates and particularly *Escherichia* spp., *Salmonella* spp., *Pseudomonas* spp., and *Acinetobacter* spp. represented 46% of all tested isolates (Fig. 4). For antimicrobial agents representing penicillins, potentiated penicillins and first or second generation cephalosporins, cefuroxime, ampicillin and cefalexin were tested in multiple studies. The latter three drugs had the highest cumulative frequency of resistance among Gram-negative isolates (Table 1 and Fig. 6). The median frequency and range of resistant Gram-negative isolates against cefuroxime, ampicillin and cefalexin were 75% (0-100), 53% (0-100) and 3% (0-100) respectively. There was some variation in the frequency of resistance against this class of antibiotics across different geographical locations but, a relatively low level of resistance was seen in Spain, Portugal, and Uganda (Fig. 6).

For antimicrobial agents representing third to fifth generation cephalosporins, ureidopenicillins and carbapenems, the most widely tested drugs across different geographical locations were piperacillin, aztreonam, ceftazidime, cefotaxime and cefepime (Table 1). These four specific drugs also had the highest cumulative frequency of resistance among Gram-negative isolates; the median frequency and range against piperacillin, aztreonam, ceftazidime, cefotaxime and cefepime were 62% (0-100), 12% (0-72), 11% (0-100), 6% (0-100) and 3% (0-59) respectively (Table 1, Fig. 7). Generally, the geographical clustering of high incidences of resistance against beta-lactams was in wastewater from livestock slaughterhouses in Nigeria, India, Turkey, and Iran, as well as poultry and pig slaughterhouses in Brazil and Germany (Fig. 7).

In the aminoglycoside class, streptomycin, kanamycin, and gentamicin were widely tested across different locations; these specific antibiotics had the highest cumulative frequency of resistance among Gram-negative isolates (Table 1 and Fig. 8). The median frequency and range of resistance against streptomycin, kanamycin and gentamicin were 44% (0-84), 26% (0-100), and 8% (0-100) respectively. (Table 1). Generally, very low incidences of resistance against gentamicin or amikacin

were observed across many nations but a few exceptions were in wastewater from a pig slaughterhouse in Taiwan (100%) and, livestock slaughterhouses in Iran (100%), Nigeria (44-100%) and Ethiopia (20%) (Fig. 8). For the quinolone class, nalidixic acid and ciprofloxacin were tested at multiple geographical locations and very varied incidences of resistance were observed. The median frequency and range against nalidixic acid and ciprofloxacin were 18% (0-81) and 14% (0-93) respectively. Relatively high incidences of resistance against ciprofloxacin were associated with wastewater from livestock slaughterhouses in Iran (93%), Nigeria (50%) and China (20%) and, poultry slaughterhouses in Germany (21-81%) and Spain (56%) (Fig. 8). Other widely tested antimicrobial compounds with relatively high incidences of resistance in Gram-negative bacteria were tetracycline, trimethoprim-sulfamethoxazole, and chloramphenicol (Table 1 and Fig. 9). Incidences of resistance to tetracycline and trimethoprim-sulfamethoxazole were relatively similar across the tested sites but the highest incidences of resistance to chloramphenicol were clustered around livestock slaughterhouses in Mexico, Australia, China, and France, as well as pig slaughterhouses in Taiwan and Germany (Fig. 9).

3.3 Genetic determinants of AMR in animal slaughterhouse wastewater

Concurrent quantification of genetic drivers of the widely spread phenotypic AMR in slaughterhouse wastewater was limited. Only 17/37 (46%) of included studies genotyped bacterial isolates for genetic markers of AMR. In Gram-negative bacteria predominated by *E. coli*, genetic markers for resistance against beta-lactam drugs were the most characterised across multiple studies and locations. For genes encoding for extended-spectrum beta-lactamases (ESBL), *bla*CTX-M, *bla*TEM and *bla*CTX-M-15 were the most prevalent and the median frequency and range were 67% (33-95), 38% (9-95), and 26% (13-50) respectively (Fig 10A). Cumulative incidences for all tested ESBL genes in Gram-negative isolates were highest in wastewater from livestock slaughterhouses in China and Iran, and a veal slaughterhouse in France (Fig. 9). It is worth noting however, that the

relatively low frequency in the expression of these genes in all other represented nations may be attributed to limited testing as opposed to low rates of expression. For example, some of the phenotypic screening for AMR in wastewater from animal slaughterhouses in Germany did not screen for ESBL gene expression but the tests were based on isolates selected for ESBL phenotype using specific antibiotics. For genes encoding for resistance against non-beta-lactam drugs in at least three distinct geographical locations, median incidence and range were 37% (5-100) for *tetA*, 26% (23-55) for *sulII*, 20% (3-22) for *sulI*, 10% (8-14) for *sulIII*, 8% (6-67) for *aadA1* and, 8% (5-23) for *cmIA* (Fig. 10B). These genes encode for resistance against tetracyclines (*tetA*), sulphonamides (*sulI*, *sulII* and *sulIII*), aminoglycosides (*aadA1*), and chloramphenicol (*cmIA*). Geographically, a very high frequency in the expression of *tetA* was associated with wastewater from a livestock slaughterhouse in Iran (100%) and a veal slaughterhouse in France (66%). The highest incidence of *sulI*, *sulII* and *sulIII* expression was associated with a livestock slaughterhouse in China and a veal slaughterhouse in France. The highest incidence in the expression of *cmIA* against chloramphenicol was also associated with bacterial isolates from a veal slaughterhouse in France (23%), while the highest frequency in the expression of *aadA1* against aminoglycosides was associated with a mixed species slaughterhouse in Portugal (67%) (Fig. 10B).

4. Discussion

4.1 Geographical locations and targeted bacteria for screening resistance

In line with the primary objective of this study, we present a critical assessment of recorded incidences and distribution of AMR in effluent wastewater from abattoirs and/or animal slaughterhouses. The data presented herein were generated from a limited geographical range as shown in Fig. 2 and are thus unlikely to provide an exhaustive evaluation of the environmental impact and magnitude of this source of AMR. This is partly understandable since most general knowledge on slaughter of animals for

301 food is perceived as controversial and not made widely available for myriad reasons
302 (Algers and Berg, 2017). This fact notwithstanding, a realistic characterisation of animal
303 slaughter facilities as a source and point for environmental dissemination of AMR
304 requires more rigorous studies, particularly from North and South America, Australasia,
305 and Eastern Europe. In addition, for all collated studies in represented regions, there was
306 marked heterogeneity and insufficient characterisation of many parameters such as
307 sample size and sampling strategy, capacity of animal slaughter facility, wastewater
308 treatment and disposal processes, and the specific bacteria targeted for screening. All
309 these parameters are critically important for the quantification of AMR from wastewater
310 sources, and these factors are discussed in previous reviews (Hassoun-Kheir et al., 2020;
311 Nguyen et al., 2021). Culture of specific bacteria followed by antibiotic sensitivity testing
312 was the main method used in all studies included in this review (Supplemental Table 1).
313 This represents majority of studies in this area, and it is advantageous because of the
314 direct link to quantifiable functional phenotypic outcomes. However, culture and
315 sensitivity testing has some limitations given that less than 10% of bacteria in each
316 microbiome can be cultured under laboratory conditions and over 99% of 10^{30} microbial
317 cells on earth are thought to be unculturable (Tarnecki et al., 2017; Johny et al., 2021).
318 Thus, targeted culturing of specific bacteria is likely to represent only a small fraction of
319 the microbiome in an environmental sample. Moreover, reports on AMR in the
320 environment do not use standardised cut-off values and results are based on Clinical
321 Laboratory Standards Institute guidelines or European Committee on Antimicrobial
322 Susceptibility Testing values. These limitations could be addressed by focussing on more
323 robust techniques such as metagenomic detection of ARGs and bacteria in future studies.
324 We show that most of the reported culture and sensitivity screening targeted *Escherichia*
325 spp., *Enterococcus* spp. and MRSA. These bacterial species are abundant in the

gastrointestinal systems of domestic and wild animals, but they do not represent the diversity in gut bacteria likely to contaminate effluent water from animal slaughter facilities. More importantly, these bacterial species have a potential to carry disease-causing virulence factors in specific strains and, may foster interspecies horizontal transfer of genetic markers of AMR between pathogenic and non-pathogenic commensal species as has been suggested for some mammalian gastrointestinal system Enterobacteriaceae (Stecher et al., 2012). A comprehensive understanding of wastewater from animal slaughter facilities as a source for environmental contamination with AMR, will require metagenomic analyses to characterise entire resistomes (ARGs and all associated bacteria) to supplement phenotypic characterisations of AMR.

4.2 Resistance factors for Beta-lactams, tetracyclines and sulphonamides

In all represented Gram-positive and Gram-negative bacteria, the highest frequency and most widely distributed AMR was against penicillins, first to second generation cephalosporins, tetracycline and sulphonamides (Fig. 5, 6 and 9). This observation is not surprising and may be mostly attributed to the fact that these antimicrobial classes are commonly applied as first-line agents for improved feed conversion, growth promotion or disease prevention in livestock and poultry across many geographical locations (Van Boeckel et al., 2015; ECDC/EFSA/EMA, 2015). For instance, in the European Union (EU) represented by many studies included herein, tetracyclines, penicillins and sulphonamides account for close to 70% of all antimicrobial drugs sold, with a similar trend seen globally (Scott et al., 2018; Jane et al., 2021). In contrast, we observed a variable but relatively high frequency of resistance against third to fifth generation cephalosporins, ureidopenicillins and carbapenems such as ceftazidime, cefotaxime, cefepime, ceftaroline, piperacillin and aztreonam in some

specific locations. This too was not surprising since most tests against these specific drugs targeted Gram-negative bacteria expressing the ESBL phenotype, suggesting a possibility of intrinsic as well as acquired resistance (Tewari et al., 2022; Aurilio et al., 2022). Nonetheless, the presence of a high frequency of this type of AMR in animal slaughterhouse effluent intended for release to the environment is concerning given the extensive use of these beta-lactams in the medical management of life-threatening, multi-drug resistant, Gram-negative bacterial infections caused by pathogens such as *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and pathogenic *E. coli* (WHO, 2017). There is no evidence directly linking AMR caused by ESBL in slaughterhouse effluents with increased livestock or poultry farm consumption of these antibiotics. However, recent trends suggest increased antibiotic usage as a likely driver. Increased occurrence of ESBL-producing *E. coli* were found to strongly correlate with higher consumption of third to fifth generation cephalosporins in conventional Dutch and Danish pig farms (Hammerum et al., 2014; Dohmen et al., 2017), suggesting some selection pressure driven by exposure to specific antibiotics.

4.3 Resistance factors for Quinolones, aminoglycosides and other reserved antibiotics

Among other reserved, non-beta-lactam antimicrobial classes, a sparser pattern with variable low to high incidences of resistance was seen with macrolides and lincosamides (vancomycin and clindamycin), aminoglycosides (gentamicin and amikacin), quinolones (nalidix acid, ciprofloxacin and enrofloxacin), chloramphenicol, nitrofurantoin, rifampicin, colistin and quinupristin-dalfopristin (Fig. 8 and 9). As observed for ESBLs, the variability in incidences appeared to be associated with the extent of antibiotic use during livestock or poultry farming. In Uganda for example, the

376 very low frequency of resistance against many of the tested antibiotics could have been
377 accidental due to targeting very rare and non-pathogenic *Salmonella* spp in pigs and
378 ruminants for AMR screening. However, this observation is more likely to be attributed
379 to the relatively nominal usage of antibiotics on livestock farms which are predominantly
380 subsistence agro-pastoral, ranches or semi-intensive in nature. To substantiate this, a
381 related but independent study reported very low incidences of phenotypic or genotypic
382 resistance against fluoroquinolones, aminoglycosides, and chloramphenicol in livestock
383 in Uganda (Okubo et al., 2019). In marked contrast, we detected relatively high
384 incidences of resistance to macrolides, fluoroquinolones, aminoglycosides, or
385 chloramphenicol in Iran, India, Nigeria, China, Taiwan, and a few countries in the EU
386 including Portugal, Spain, France, and Germany. Interestingly, our observations here do
387 coincide with some global estimates that have ranked China (45%), Brazil (7.9%), USA
388 (7.0%), Thailand (4.2%), India (2.2%), Iran (1.9%), Spain (1.9%), Russia (1.8%), Mexico
389 (1.7%), and Argentina (1.5%), among top ten countries with the largest antibiotic
390 footprint (% of global consumption) in agriculture and veterinary medicine (Tiseo, 2020).
391 Nigeria also showed very high incidences of AMR in our evaluations; it has been ranked
392 among the top 50 countries with the largest antimicrobial use in livestock with a
393 projected 163% increase by 2030 (Van Boeckel et al., 2015). More specifically, it has
394 been estimated that fluoroquinolones and aminoglycosides may account for 27% and
395 20% of all antibiotics used in livestock farming in Nigeria respectively (Adesokan et al.,
396 2015). These statistics seem to match the relatively high incidences of resistance against
397 gentamicin, kanamycin, and ciprofloxacin that we observed in wastewater from animal
398 slaughter facilities in Nigeria. Collectively, these are troubling trends since many of these
399 specific antimicrobial agents are mostly reserved for multi-drug resistant bacterial
400 infections in jurisdictions with stricter antibiotic stewardship programs (Van Boeckel et

al., 2015). In humans and most animals, fluoroquinolones such as ciprofloxacin and enrofloxacin have favourable pharmacokinetic features with very good distribution in most tissues, a concentration-dependent bactericidal activity, and long terminal half-lives (Wispelwey, 2005). Fluoroquinolones also have proven clinical efficacy for many types of infections including community-acquired pneumonia in humans and urinary as well as respiratory tract infections in veterinary patients and there is a well-established link between enrofloxacin use in poultry and fluoroquinolone resistance among *Campylobacter* isolates in humans (Wispelwey, 2005; Weese et al., 2015). Equally important are chloramphenicol and its derivatives, macrolides, and rifampicin that have good pulmonary distribution, ability to concentrate in macrophages and penetrate granulomas. These drugs are ideal for respiratory tract infections caused by intracellular bacteria associated with granulomatous reactions such as *Rhodococcus equi* in horses and *Mycobacteria* spp. in humans (Wispelwey, 2005; Abulfathi et al., 2019). Indiscriminate, unregulated use of all these antimicrobial compounds is likely to lead to unmitigated widespread resistance; it should be avoided. Despite the likely immediate short-term costs, withdrawal of some classes of antibiotics in food-producing animals can be associated with a subsequent decline in resistance profiles in humans and animals (Nilsson, 2012). Implementation of corrective measures will be dictated by carefully crafted policies and antibiotic stewardship programs in the different geo-political jurisdictions. An example of this is the World Health Organisation and the World Organisation for Animal Health recommendations that guided the Australian Strategic and Technical Advisory Group on Antimicrobial Resistance to prioritise and limit the use of all critically reserved classes of antibiotics (Office of Health Protection, Commonwealth of Australia, 2018).

4.4 Other general determinants of AMR in animal slaughterhouse

wastewater

It is established that the rate and scale of antibiotic use in poultry and livestock farms does differentially select for AMR (Holmes et al., 2016; Burows and Kasbohrer, 2017); this notion is supported by summarised data in the current study. Identified phenotypic AMR patterns are discussed above, but detailed molecular and genetic mechanisms that underlie these manifestations are mostly unknown. Very limited genotyping for biomarkers of AMR in animal slaughterhouse effluents has been done to date; this limited our ability to perform any in-depth analyses in the current study. The limited available data indicates that similar and commonly identifiable genetic determinants of AMR (McEwen and Collignon, 2018; Chen et al., 2020; de Abreu et al., 2021), are involved in this human-animal-environment interface as well. Conventional ESBL encoding genes *bla*CTX-M, *bla*TEM and *bla*CTX-M-15, were the most prevalent in beta-lactam resistant isolates, while *tetA*, *aadA1* and *cmIA* were associated with resistance against tetracyclines, aminoglycosides and chloramphenicol respectively (Fig. 10). The detection of class 1 integron (*int1*) in slaughterhouse effluents in Nigeria and France indicates a possibility of horizontal gene transfer and the amplification of AMR in these environmental matrices since this gene encodes for recombinases that can excise and insert exogenous multi-resistance gene cassettes into bacterial chromosomes or plasmids (Gillings, 2014). While these data are limited in scope, cumulative incidences, and distribution of identified genetic markers of AMR appear to follow similar trends as observed for phenotypic AMR patterns (section 3.3). This apparent congruence on prevalence and distribution of genetic and phenotypic markers of AMR in slaughterhouse effluents is purely observational and has not been tested statistically. Future studies on this topic should confirm these associations.

Different farming systems, environments, and jurisdictional policies on antibiotic usage are likely to differentially drive the development of phenotypic and/or genotypic AMR (Landers et al. 2012; Van Boeckel et al., 2015), that is ultimately detectable in effluent wastewater from animal slaughter

facilities worldwide as shown in the current study. The United Nations Department of Economic and Social Affairs predicts that the world's human population will get to 9.8 billion in 2050 (United Nations, 2017). This will create a huge demand for agricultural and other livestock products and thus, drive the intensification of farming systems and methods. Intensive livestock and poultry farms are associated with the regular use of antibiotics, often in subtherapeutic doses for disease prevention and growth promotion as opposed to treating diseases (Van Boeckel et al., 2015; Silbergeld et al., 2008). In extracted data for this review, one study showed that even within the same country, slaughterhouses that were dedicated to free range poultry farms had a much lower incidence of resistance against all major antimicrobial classes, compared to slaughterhouses for conventional intensive poultry farms (Da Costa et al., 2006). A two-fold higher incidence of resistance against ampicillin, cefalexin, streptomycin, trimethoprim-sulfamethoxazole, enrofloxacin, chloramphenicol, gentamicin and kanamycin were observed in the conventional poultry systems as compared to a free-range system (Supplemental Table 1). This finding also highlights a need for regulatory action that is, ideally, internationally coordinated to avoid the predicted consequences of increased AMR in humans, animals, and the environment. The framing and execution of these regulatory actions is a critically important topic, but one that is beyond the limited scope of the current review. It is worth noting however, that in any jurisdiction, the scale of antibiotic use and the prevalence of AMR, tends to be dictated by economic or market conditions, and social as well as cultural norms (Robles-Jimenez et al., 2021). This argument is supported by some observations in the current study and some specific examples are informative. It has been reported that some countries in continental Asia lack or have not implemented policies to regulate the use of antibiotics in the livestock sector (Landers et al., 2012; Sakeena et al., 2018). We show that this coincides with high incidences of AMR in animal slaughterhouse effluents in parts of China, India, Iran, Turkey, and Malaysia (Fig. 5-9). On the other hand, the EU presents a different but interesting scenario. General use of antibiotics for veterinary or human clinical applications is restricted and closely monitored in many EU

countries. In some EU member countries including but not limited to Portugal, Spain, Italy, and Germany however, the use of relatively higher quantities of antibiotics in intensive livestock or poultry farming is still common (Van Boeckel et al., 2015; European Medicines Agency, 2019). Coincidentally, we show the presence of a relatively high incidence of AMR in animal slaughterhouse wastewater in Portugal, Spain, and Germany.

Other important considerations are the spatial and temporal distributions of AMR bacteria associated with animal slaughterhouse wastewater; this information would seem to be a pre-requisite in designing mitigation strategies. We have characterised the spatial distribution of this problem from the limited existing literature. However, in all studies that qualified for this review, temporal distribution patterns were poorly defined and as a result, it was difficult to critically evaluate how AMR varied seasonally. Seasonality should be a strong consideration in designing future studies on this topic. In addition, the treatment, processing, and disposal of farm and slaughterhouse wastewater is a critical determinant in the magnitude of environmental pollution with AMR bacteria. Collated data for this study shows that any formal processing of wastewater from animal slaughterhouse facilities may lower incidences of AMR compared to untreated wastewater (Supplemental Fig. 1). However, these differences were not tested statistically given the marked heterogeneity and lack of documentation in slaughterhouse wastewater treatment and disposal processes. It is possible therefore, that formal and extensive wastewater processing for some of the represented animal slaughterhouses was not captured in our analyses. Consequently, we recommend that detailed descriptions of relevant wastewater treatment processes if any, should be incorporated in future technical reports, policy documents or peer reviewed publications on this topic. It remains critically important to determine and characterise all epidemiological associations between effluents from food animal slaughter facilities and the environmental dissemination of

AMR internationally. Concerted global studies on this human-animal-environmental problem from a One Health perspective are warranted.

5. Conclusions

To our knowledge, this is the first study to provide a critical assessment of AMR patterns in wastewater effluents from food animal slaughter facilities via a systematic evaluation of existing literature. Phenotypic AMR via culture and sensitivity testing was detected against all the major antimicrobial groups including third to fifth generation cephalosporins, fluoroquinolones, aminoglycosides, macrolides, and chloramphenicol. The prevalence and spatial global distribution of AMR in animal slaughterhouse wastewater was highly variable and appeared to depend on several apparent factors including: frequency and magnitude of antibiotic usage in livestock or poultry farms, animal species and the scale of farming systems involved, treatment of slaughterhouse wastewater prior to disposal in the environment, and the existence of implementable policies on the regulation of antibiotic use in stewardship programs in the different jurisdictions. These observations indicate that anthropogenic activities in farming systems for food animals, are a major contributor to the dissemination of AMR bacteria and ARG in the environment via slaughterhouse wastewater effluents. Along the One Health continuum, these determinants of AMR are bound to recycle in humans, livestock and other aquatic or terrestrial organisms.

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Author Contribution Statement

Leo Foyle: Conceptualisation, methods, data curation and analysis, supervision, reviewing and editing. Matthew Burnett, Abbey Creaser, Rachel Hens, Julia Keough, Lauren Madin, Ruby Price, Hayley Smith & Samuel Stone: These authors contributed equally to methods, data curation and analysis, and writing the initial manuscript draft. Robert T. Kinobe: Conceptualisation, methods, data curation and analysis, supervision, writing, reviewing and editing.

Declaration of competing interest

All authors declare that they have no competing financial or personal interests or relationships that could influence the work reported in this paper.

Data availability

All data associated with this paper are included herein or have been provided as supplementary material.

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949 **Table 1. Cumulative Median and range of AMR (%), and number of studies for Gram-negative isolates**

Penicillins, 1st/2nd Gen.	3rd/4th Generation	Aminoglycosides,	
Cephalosporins	Cephalosporins,	Quinolones &	
	Ureidopenicillins and	Macrolides	
	Carbapenems		
Penicillins	Cephalosporins	Aminoglycosides	Sulphonamides and
			Tetracyclines
PEN 100 (<i>n</i> = 1)	CFM 62 (24-100, <i>n</i> = 2)	STR 44 (0-84, <i>n</i> = 13)	SDZ 62 (<i>n</i> = 1)
AMP 53 (0-100, <i>n</i> = 14)	CPD 32 (<i>n</i> = 1)	KAN 26 (0-100, <i>n</i> = 8)	SXT 36 (4-52, <i>n</i> = 12)
AMX 29 (<i>n</i> = 1)	CZA 11 (1-21, <i>n</i> = 2)	GEN 8 (0-100, <i>n</i> = 20)	SMX 18 (<i>n</i> = 1)
AMC 3 (0-100, <i>n</i> = 11)	CAZ 11 (0-100, <i>n</i> = 14)	APR 2 (<i>n</i> = 1)	TET 52 (0-100, <i>n</i> = 19)
	CTX 6 (0-100, <i>n</i> = 15)	TOB 0 (<i>n</i> = 1)	TGC 0 (0-94, <i>n</i> = 3)
	CRO 6 (0-27, <i>n</i> = 4)	AMK 1 (0-60, <i>n</i> = 10)	
	FEP 3(0-59, <i>n</i> = 8)		
Cephalosporins	Ureidopenicillins and	Quinolones	Other classes
	Carbapenems		
CXM 75 (0-100, <i>n</i> = 9)	PIP 62 (0-100, <i>n</i> = 7)	NAL 18 (0-81, <i>n</i> = 14)	NIT 16 (3-50, <i>n</i> = 5)
CFZ 18 (<i>n</i> = 1)	CAR 51 (<i>n</i> = 1)	ENR 17 (<i>n</i> = 1)	Q-D 15 (<i>n</i> = 1)
CEC 18 (<i>n</i> = 1)	ATM 12 (0-72, <i>n</i> = 8)	CIP 14 (0-93, <i>n</i> = 22)	CHL 12 (0-62, <i>n</i> = 13)
FOX 5 (0-50, <i>n</i> = 7)	ETP 3 (2-100, <i>n</i> = 3)	MXF 10 (4-52, <i>n</i> = 3)	RIF 12 (1-24, <i>n</i> = 4)
CEF 3 (0-100, <i>n</i> = 10)	MEM 3 (0-78, <i>n</i> = 3)	LVX 10 (0-13, <i>n</i> = 3)	CST 5 (0-36, <i>n</i> = 7)
	SAM 3 (0-6, <i>n</i> = 2)	NOR 8 (0-80, <i>n</i> = 3)	VAN 1 (0-28, <i>n</i> = 3)
		Macrolides	
	IPM 0 (0-8, <i>n</i> = 8)	ERY 44 (14-77, <i>n</i> = 6)	
	TZP 1 (0-7, <i>n</i> = 6)	CLI 26 (18-100, <i>n</i> = 3)	
	C/T 7 (4-11, <i>n</i> = 3)		

950
951 Abbreviations are defined as: PEN = penicillin; AMP = ampicillin, AMX = amoxicillin; AMC = amoxicillin-clavulanic
952 acid; CXM = cefuroxime; CFZ = cefazolin; FOX = cefoxitin; CEF = cefalotin; CFM = cefixime; CPD = cefpodoxime;
953 CAZ = ceftazidime; CZA = ceftazidime-avibactam; CTX = cefotaxime; CRO = ceftriaxone; PIP = piperacillin; CAR =
954 carbenicillin; ATM = aztreonam; ETP = ertapenem; MEM = meropenem; SAM = ampicillin-sulbactam; IMP =
955 imipenem; TZP = piperacillin-tazobactam; C/T = ceftolozane-tazobactam; STR = streptomycin; KAN = kanamycin;
956 GEN = gentamicin; APR = apramycin; TOB = tobramycin; AMK = amikacin; NAL = nalidixic acid; ENR =
957 enrofloxacin; CIP = ciprofloxacin; MXF = moxifloxacin LVX = levofloxacin; NOR = norfloxacin; ERY = erythromycin;
958 CLI = clindamycin; SDZ = sulfadiazine; SXT = trimethoprim-sulfamethoxazole; SMX = sulfamethoxazole; TET =
959 tetracycline; TGC = tigecycline; NIT = nitrofurantoin; Q-D = quinupristin-dalfopristin; CHL = chloramphenicol; RIF =
960 rifampicin; CST = colistin; VAN = vancomycin.

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Figure legends

Fig. 1. An outline of the process that was used for the literature search and screening for eligibility of included studies. Thirty-seven publications met the qualitative assessment for inclusion but only 27 publications were considered for secondary analyses.

Fig. 2. Global location and clustering of documented primary studies on antimicrobial resistance in effluent wastewater from animal slaughter facilities. The cumulative frequency of animal slaughter facilities from each geographical location are represented by numbers. Blue stars indicate locations with studies that met the inclusion criteria while red dots represent locations with studies on animal slaughter facilities that did not meet the inclusion criteria and were not included in analyses. The original map was modified from Google maps under the terms and conditions of the Creative Commons Attribution (CC BY license).

Fig. 3. An illustrative scheme to show all studies that met the inclusion criteria for data extraction and the evaluation against each critical qualitative measure. Cumulative qualitative scores for each study were evaluated as percentages (bottom row) and the numbers on the right column represent the fraction of studies that met each qualitative measure.

Fig. 4. Cumulative frequency of bacterial isolates that were targeted for phenotypic or genotypic screening for antimicrobial resistance in wastewater effluent from animal slaughter facilities in all studies that met the inclusion criteria and, stratified by bacterial species among Gram-negative as well as Gram-positive strains. MRSA refers to Methicillin-resistant *Staphylococcus aureus*.

Fig. 5. Frequency and distribution of phenotypic antimicrobial resistance stratified by geographical location against commonly used beta-lactam drugs (A) and other antimicrobial classes (B), in all tested Gram-positive isolates. Abbreviations are defined as: PEN = penicillin; AMP = ampicillin, AMC = amoxicillin-clavulanic acid; CXM = cefuroxime; DCX = dicloxacillin; FOX = ceftazidime; OXA = oxacillin; MEM = meropenem; CAZ = ceftazidime; CRO = ceftriaxone; CPT = ceftaroline; CFZ = cefazolin; AMX = amoxicillin; ERY = erythromycin; TMP = TMP = trimethoprim; TET = tetracycline; NOR = norfloxacin; CIP = ciprofloxacin; KAN = kanamycin; VAN = vancomycin; NIT = nitrofurantoin; RIF = rifampicin; Q-D = quinupristin-dalfopristin; CHL = chloramphenicol; GEN = gentamicin; CLI = clindamycin; SXT = trimethoprim-sulfamethoxazole; MXF = moxifloxacin.

Fig. 6. Frequency and distribution of phenotypic antimicrobial resistance stratified by geographical location against commonly used penicillins and, first or second generation cephalosporins in all tested Gram-negative isolates. Abbreviations are defined as: AMP = ampicillin, AMC = amoxicillin-clavulanic acid; CEF = cefalotin; CXM = cefuroxime; FOX = ceftiofur; CFZ = cefazolin; CEC = cefaclor; AMX = amoxicillin; PEN = penicillin.

Fig. 7. Frequency and distribution of phenotypic antimicrobial resistance stratified by geographical location against carbapenems and third to fifth generation cephalosporins in all tested Gram-negative bacterial isolates. Abbreviations are defined as: CTX = cefotaxime; CAZ = ceftazidime; FEP = cefepime; ATM = aztreonam; SAM = ampicillin-sulbactam; CZA = ceftazidime-avibactam; MEM = meropenem; IPM = imipenem; PIP = piperacillin; ETP = ertapenem; TZP = piperacillin-tazobactam; CPD = cefpodoxime; CRO = ceftriaxone; CFM = cefixime; C/T = ceftolozane-tazobactam; CAR = carbenicillin.

Fig. 8. Frequency and distribution of phenotypic antimicrobial resistance stratified by geographical location against fluoroquinolones, aminoglycosides, macrolides and lincosamides in all tested Gram-negative isolates. Abbreviations are defined as: CIP = ciprofloxacin; NAL = nalidixic acid; AMK = amikacin; MXF = moxifloxacin; LVX = levofloxacin; NOR = norfloxacin; CLI = clindamycin; ENR = enrofloxacin; GEN = gentamicin; STR = streptomycin; ERY = erythromycin; KAN = kanamycin; APR = apramycin; TOB = tobramycin.

Fig. 9. Frequency and distribution of phenotypic antimicrobial resistance stratified by geographical location against other commonly used antibiotics in all tested Gram-negative isolates. Abbreviations are defined as: SDZ = sulfadiazine; SXT = trimethoprim-sulfamethoxazole; SMX = sulfamethoxazole; TET = tetracycline; TGC = tigecycline; NIT = nitrofurantoin; Q-D = quinupristin-dalfopristin; CHL = chloramphenicol; RIF = rifampicin; CST = colistin; VAN = vancomycin.

Fig. 10. Frequency and distribution of genotypic determinants of antimicrobial resistance as stratified by geographical location and specific genes encoding for extended spectrum beta-lactam resistance (A), and cumulative incidences of resistance against specific genetic determinants of resistance against other major antimicrobial drugs in studied geographical locations (B).

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258

259 **Table 1. Cumulative Median and range of AMR (%), and number of studies for Gram-negative isolates**

Penicillins, 1st/2nd Gen.	3rd/4th Generation	Aminoglycosides,	
Cephalosporins	Cephalosporins,	Quinolones &	
	Ureidopenicillins and	Macrolides	
	Carbapenems		
Penicillins	Cephalosporins	Aminoglycosides	Sulphonamides and
			Tetracyclines
PEN 100 (<i>n</i> = 1)	CFM 62 (24-100, <i>n</i> = 2)	STR 44 (0-84, <i>n</i> = 13)	SDZ 62 (<i>n</i> = 1)
AMP 53 (0-100, <i>n</i> = 14)	CPD 32 (<i>n</i> = 1)	KAN 26 (0-100, <i>n</i> = 8)	SXT 36 (4-52, <i>n</i> = 12)
AMX 29 (<i>n</i> = 1)	CZA 11 (1-21, <i>n</i> = 2)	GEN 8 (0-100, <i>n</i> = 20)	SMX 18 (<i>n</i> = 1)
AMC 3 (0-100, <i>n</i> = 11)	CAZ 11 (0-100, <i>n</i> = 14)	APR 2 (<i>n</i> = 1)	TET 52 (0-100, <i>n</i> = 19)
	CTX 6 (0-100, <i>n</i> = 15)	TOB 0 (<i>n</i> = 1)	TGC 0 (0-94, <i>n</i> = 3)
	CRO 6 (0-27, <i>n</i> = 4)	AMK 1 (0-60, <i>n</i> = 10)	
	FEP 3(0-59, <i>n</i> = 8)		
Cephalosporins	Ureidopenicillins and	Quinolones	Other classes
	Carbapenems		
CXM 75 (0-100, <i>n</i> = 9)	PIP 62 (0-100, <i>n</i> = 7)	NAL 18 (0-81, <i>n</i> = 14)	NIT 16 (3-50, <i>n</i> = 5)
CFZ 18 (<i>n</i> = 1)	CAR 51 (<i>n</i> = 1)	ENR 17 (<i>n</i> = 1)	Q-D 15 (<i>n</i> = 1)
CEC 18 (<i>n</i> = 1)	ATM 12 (0-72, <i>n</i> = 8)	CIP 14 (0-93, <i>n</i> = 22)	CHL 12 (0-62, <i>n</i> = 13)
FOX 5 (0-50, <i>n</i> = 7)	ETP 3 (2-100, <i>n</i> = 3)	MXF 10 (4-52, <i>n</i> = 3)	RIF 12 (1-24, <i>n</i> = 4)
CEF 3 (0-100, <i>n</i> = 10)	MEM 3 (0-78, <i>n</i> = 3)	LVX 10 (0-13, <i>n</i> = 3)	CST 5 (0-36, <i>n</i> = 7)
	SAM 3 (0-6, <i>n</i> = 2)	NOR 8 (0-80, <i>n</i> = 3)	VAN 1 (0-28, <i>n</i> = 3)
		Macrolides	
	IPM 0 (0-8, <i>n</i> = 8)	ERY 44 (14-77, <i>n</i> = 6)	
	TZP 1 (0-7, <i>n</i> = 6)	CLI 26 (18-100, <i>n</i> = 3)	
	C/T 7 (4-11, <i>n</i> = 3)		

260

261 Abbreviations are defined as: PEN = penicillin; AMP = ampicillin, AMX = amoxicillin; AMC = amoxicillin-clavulanic
 262 acid; CXM = cefuroxime; CFZ = cefazolin; FOX = cefoxitin; CEF = cefalotin; CFM = cefixime; CPD = cefpodoxime;
 263 CAZ = ceftazidime; CZA = ceftazidime-avibactam; CTX = cefotaxime; CRO = ceftriaxone; PIP = piperacillin; CAR =
 264 carbenicillin; ATM = aztreonam; ETP = ertapenem; MEM = meropenem; SAM = ampicillin-sulbactam; IMP =
 265 imipenem; TZP = piperacillin-tazobactam; C/T = ceftolozane-tazobactam; STR = streptomycin; KAN = kanamycin;
 266 GEN = gentamicin; APR = apramycin; TOB = tobramycin; AMK = amikacin; NAL = nalidixic acid; ENR =
 267 enrofloxacin; CIP = ciprofloxacin; MXF = moxifloxacin LVX = levofloxacin; NOR = norfloxacin; ERY = erythromycin;
 268 CLI = clindamycin; SDZ = sulfadiazine; SXT = trimethoprim-sulfamethoxazole; SMX = sulfamethoxazole; TET =
 269 tetracycline; TGC = tigecycline; NIT = nitrofurantoin; Q-D = quinupristin-dalfopristin; CHL = chloramphenicol; RIF =
 270 rifampicin; CST = colistin; VAN = vancomycin.

271

Figure legends

Fig. 1. An outline of the process that was used for the literature search and screening for eligibility of included studies. Thirty-seven publications met the qualitative assessment for inclusion but only 27 publications were considered for secondary analyses.

Fig. 2. Global location and clustering of documented primary studies on antimicrobial resistance in effluent wastewater from animal slaughter facilities. The cumulative frequency of animal slaughter facilities from each geographical location are represented by numbers. Blue stars indicate locations with studies that met the inclusion criteria while red dots represent locations with studies on animal slaughter facilities that did not meet the inclusion criteria and were not included in analyses. The original map was modified from Google maps under the terms and conditions of the Creative Commons Attribution (CC BY license).

Fig. 3. An illustrative scheme to show all studies that met the inclusion criteria for data extraction and the evaluation against each critical qualitative measure. Cumulative qualitative scores for each study were evaluated as percentages (bottom row) and the numbers on the right column represent the fraction of studies that met each qualitative measure.

Fig. 4. Cumulative frequency of bacterial isolates that were targeted for phenotypic or genotypic screening for antimicrobial resistance in wastewater effluent from animal slaughter facilities in all studies that met the inclusion criteria and, stratified by bacterial species among Gram-negative as well as Gram-positive strains. MRSA refers to Methicillin-resistant *Staphylococcus aureus*.

Fig. 5. Frequency and distribution of phenotypic antimicrobial resistance stratified by geographical location against commonly used beta-lactam drugs (A) and other antimicrobial classes (B), in all tested Gram-positive isolates. Abbreviations are defined as: PEN = penicillin; AMP = ampicillin, AMC = amoxicillin-clavulanic acid; CXM = cefuroxime; DCX = dicloxacillin; FOX = cefoxitin; OXA = oxacillin; MEM = meropenem; CAZ = ceftazidime; CRO = ceftriaxone; CPT = ceftaroline; CFZ = cefazolin; AMX = amoxicillin; ERY = erythromycin; TMP = TMP = trimethoprim; TET = tetracycline; NOR = norfloxacin; CIP = ciprofloxacin; KAN = kanamycin; VAN = vancomycin; NIT = nitrofurantoin; RIF = rifampicin; Q-D = quinupristin-dalfopristin; CHL = chloramphenicol; GEN =

gentamicin; CLI = clindamycin; SXT = trimethoprim-sulfamethoxazole; MXF = moxifloxacin.

Fig. 6. Frequency and distribution of phenotypic antimicrobial resistance stratified by geographical location against commonly used penicillins and, first or second generation cephalosporins in all tested Gram-negative isolates. Abbreviations are defined as: AMP = ampicillin, AMC = amoxicillin-clavulanic acid; CEF = cefalotin; CXM = cefuroxime; FOX = cefoxitin; CFZ = cefazolin; CEC = cefaclor; AMX = amoxicillin; PEN = penicillin.

Fig. 7. Frequency and distribution of phenotypic antimicrobial resistance stratified by geographical location against carbapenems and third to fifth generation cephalosporins in all tested Gram-negative bacterial isolates. Abbreviations are defined as: CTX = cefotaxime; CAZ = ceftazidime; FEP = cefepime; ATM = aztreonam; SAM = ampicillin-sulbactam; CZA = ceftazidime-avibactam; MEM = meropenem; IPM = imipenem; PIP = piperacillin; ETP = ertapenem; TZP = piperacillin-tazobactam; CPD = cefpodoxime; CRO = ceftriaxone; CFM = cefixime; C/T = ceftolozane-tazobactam; CAR = carbenicillin.

Fig. 8. Frequency and distribution of phenotypic antimicrobial resistance stratified by geographical location against fluoroquinolones, aminoglycosides, macrolides and lincosamides in all tested Gram-negative isolates. Abbreviations are defined as: CIP = ciprofloxacin; NAL = nalidixic acid; AMK = amikacin; MXF = moxifloxacin; LVX = levofloxacin; NOR = norfloxacin; CLI = clindamycin; ENR = enrofloxacin; GEN = gentamicin; STR = streptomycin; ERY = erythromycin; KAN = kanamycin; APR = apramycin; TOB = tobramycin.

Fig. 9. Frequency and distribution of phenotypic antimicrobial resistance stratified by geographical location against other commonly used antibiotics in all tested Gram-negative isolates. Abbreviations are defined as: SDZ = sulfadiazine; SXT = trimethoprim-sulfamethoxazole; SMX = sulfamethoxazole; TET = tetracycline; TGC = tigecycline; NIT = nitrofurantoin; Q-D = quinupristin-dalfopristin; CHL = chloramphenicol; RIF = rifampicin; CST = colistin; VAN = vancomycin.

Fig. 10. Frequency and distribution of genotypic determinants of antimicrobial resistance as stratified by geographical location and specific genes encoding for extended spectrum beta-lactam resistance (A), and cumulative incidences of resistance against specific genetic determinants of resistance against other major antimicrobial drugs in studied geographical locations (B).

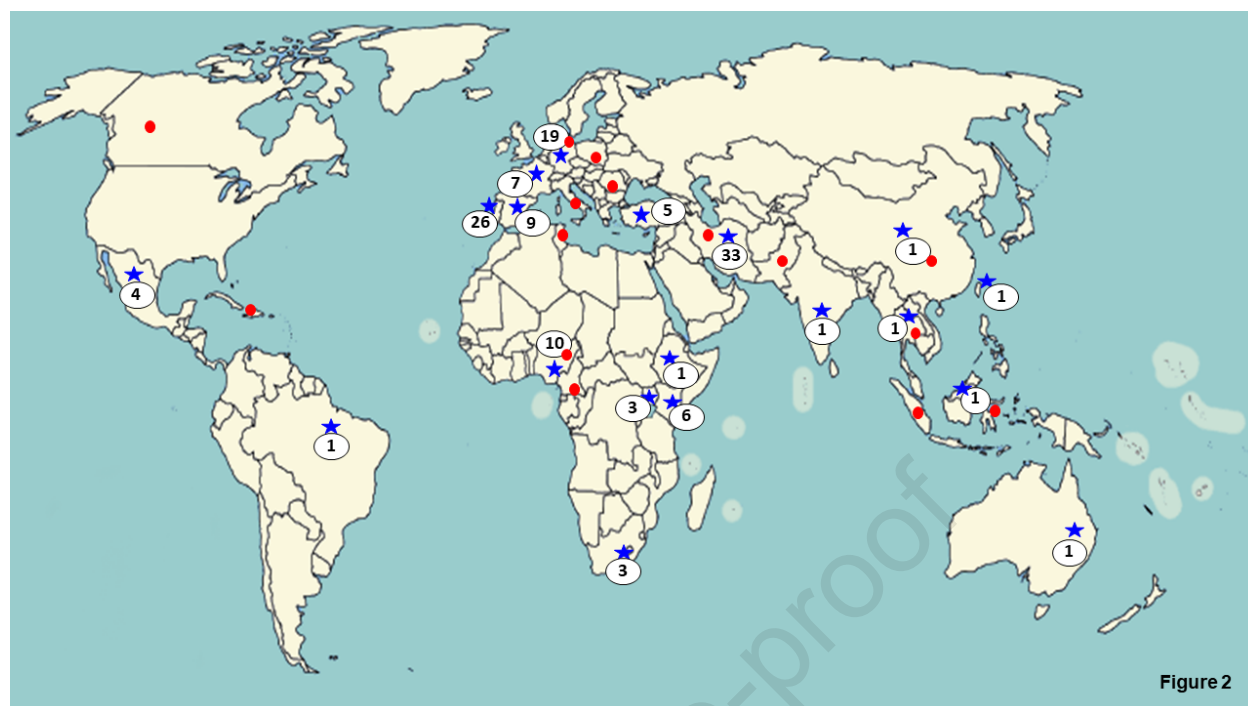


Figure 2

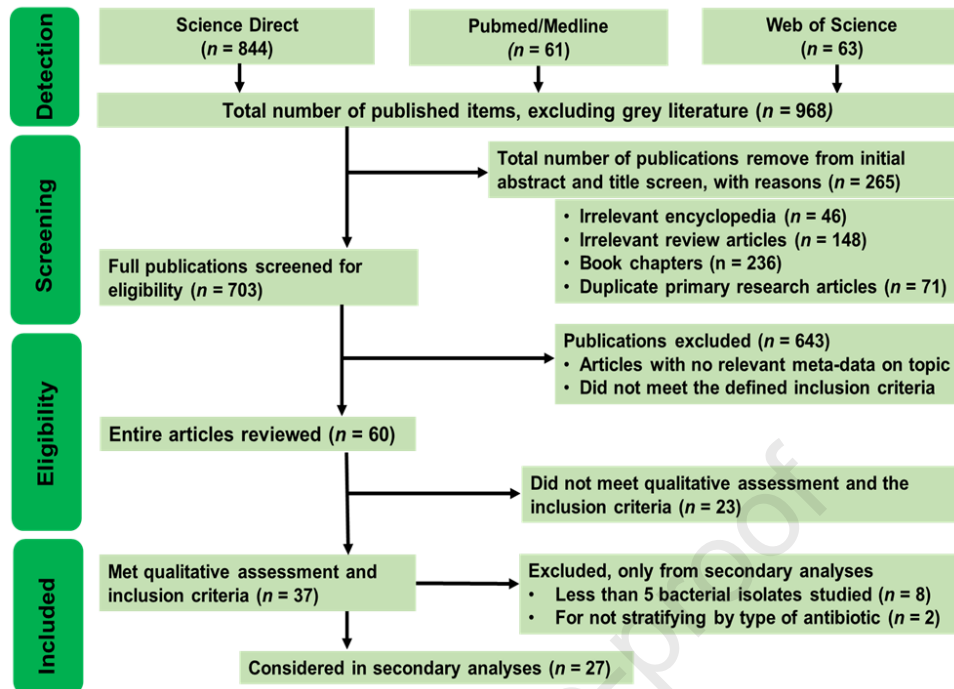


Figure 1

Figure 3

Figure 3	Savin et al. 2021	Wang et al. 2021	Talavera-G et al. 2021	Ali et al. 2021	Homeier-G et al. 2021	Serhat et al. 2021	Sib et al. 2020	Akpan et al. 2020	Naraghi et al. 2020	Olawale et al. 2020	Perez-E et al. 2020	Savin et al. 2020a	Savin et al. 2020b	Tonekabony et al. 2021	Tesfaye et al. 2019	Bolukaoto et al. 2019	Abidatul et al. 2018	Igbinosa et al. 2017	Odjadjare et al. 2017	Sudarwanto et al. 2017	Um et al. 2016	Afema et al. 2016	Dupouy et al. 2016	Ayaz et al. 2015	Wan et al. 2014	de Oliveira et al. 2013	Diallo et al. 2013	Igbinosa et al. 2012	Araujo et al. 2010	Garcia-A et al. 2009	da Costa et al. 2008	Sabate et al. 2008	Moura et al. 2007	da Costa et al. 2006	Kariuki et al. 2002	Adesiyun et al. 1989	Bensink et al. 1981				
1. Precise location in nation	X	X	X	X	X	X	X	✓	✓	✓	✓	X	X	X	✓	✓	✓	✓	✓	✓	X	✓	✓	X	✓	X	✓	X	✓	X	X	✓	✓	✓	✓	✓	✓	✓	✓	20/37	
2. Animal spp. & no. of abattoirs	✓	X	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	X	✓	✓	X	✓	X	X	X	✓	✓	✓	✓	✓	✓	✓	X	✓	✓	✓	✓	✓	X	✓	✓	✓	✓	✓	29/37
3. Abattoir capacity ± season	✓	X	X	✓	X	X	X	✓	X	X	X	✓	✓	X	✓	X	X	X	X	X	X	✓	X	✓	✓	X	✓	✓	✓	X	X	✓	X	X	✓	X	X	X	X	X	13/37
4. Defined wastewater treatment /disposal process	✓	X	X	X	X	X	X	✓	✓	✓	✓	✓	✓	✓	X	✓	X	X	✓	X	✓	✓	✓	✓	✓	X	✓	✓	✓	X	X	✓	✓	✓	✓	✓	X	✓	✓	✓	22/37
5. Defined sample number	✓	X	✓	✓	✓	✓	X	✓	✓	✓	X	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	X	X	✓	✓	✓	✓	X	✓	✓	✓	✓	31/37
6. Sampling strategy justified	✓	X	✓	X	X	X	X	X	X	X	X	✓	✓	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	✓	X	X	X	X	X	X	X	X	5/37
7. Defined AMR phenotype by individual species	✓	✓	✓	X	X	X	X	X	✓	X	X	✓	✓	✓	X	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	X	✓	✓	✓	✓	✓	28/37
8. AMR Phenotyping on > 10 effluent isolates	X	✓	✓	✓	✓	X	✓	✓	✓	✓	X	✓	✓	✓	X	X	✓	✓	✓	X	✓	✓	✓	✓	✓	✓	✓	X	✓	X	✓	✓	✓	✓	✓	✓	✓	X	X	✓	27/37
9. AMR Phenotype quantified	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	37/37
10. Some genetic mechanisms	✓	✓	✓	✓	X	X	✓	X	✓	X	✓	X	✓	✓	X	X	X	X	✓	X	✓	✓	✓	✓	✓	✓	X	X	X	X	X	X	X	✓	✓	X	X	X	X	X	17/37
Overall qualitative measure (%)	70	40	70	70	40	30	40	70	80	60	50	80	90	50	60	50	60	70	60	50	80	70	80	70	80	80	80	60	50	40	50	80	70	60	60	60	60	30			

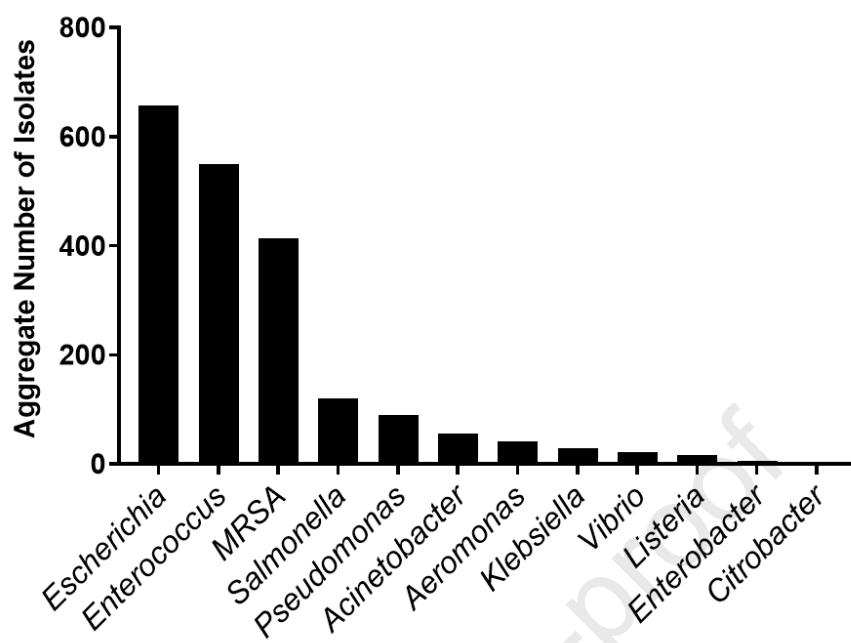


Figure 4

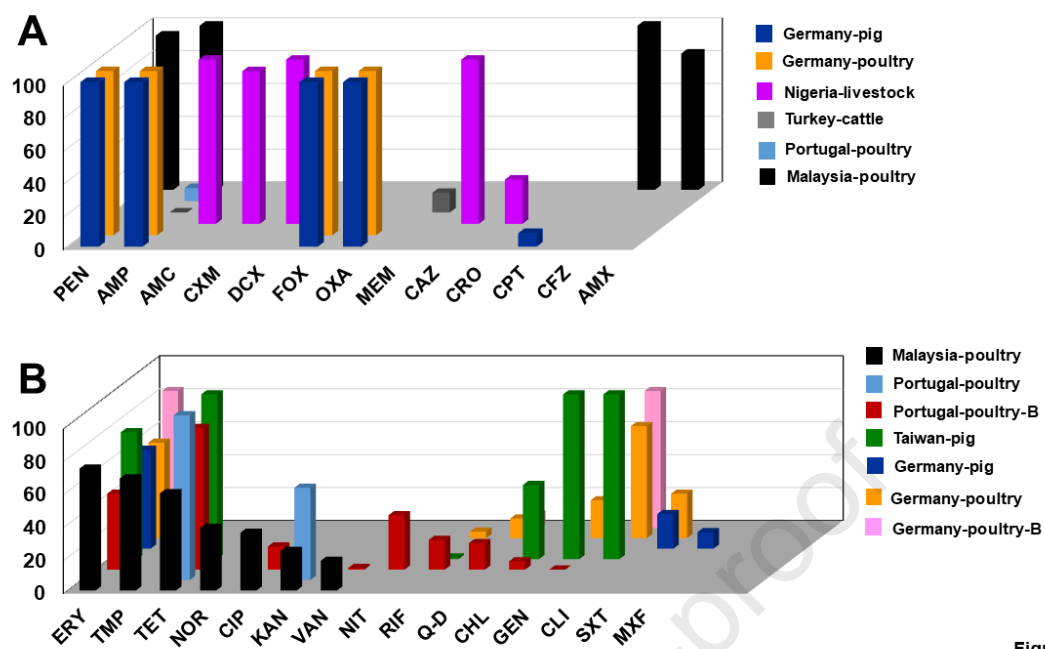


Figure 5

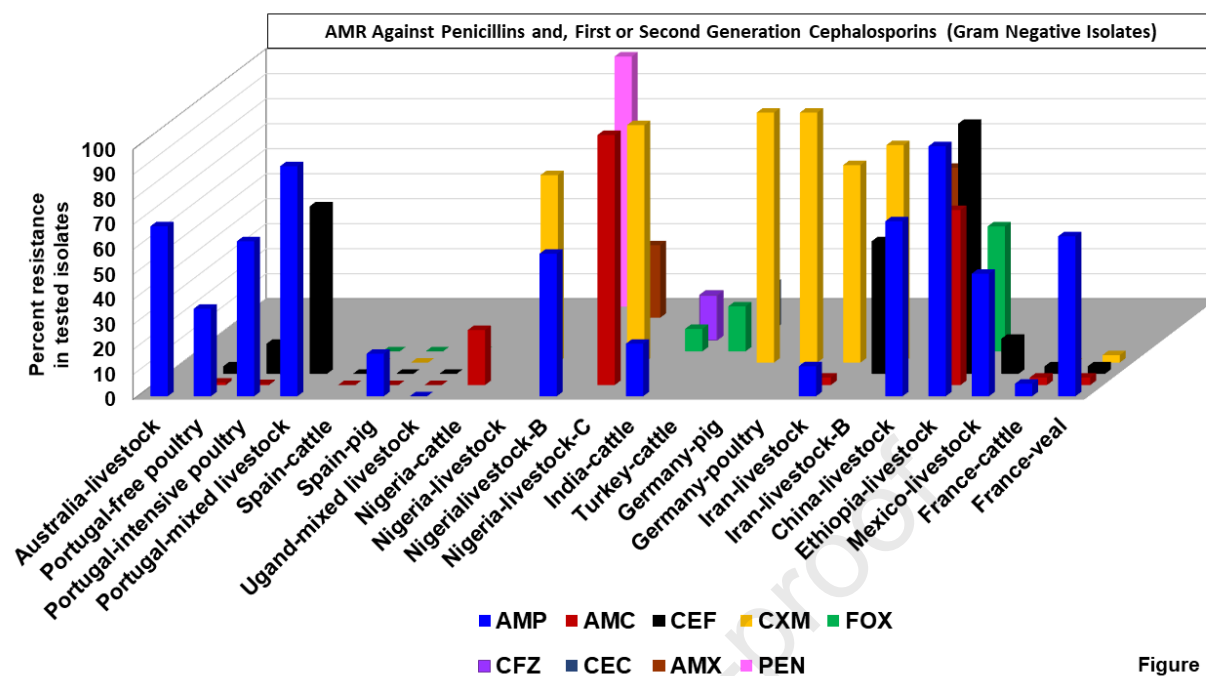


Figure 6

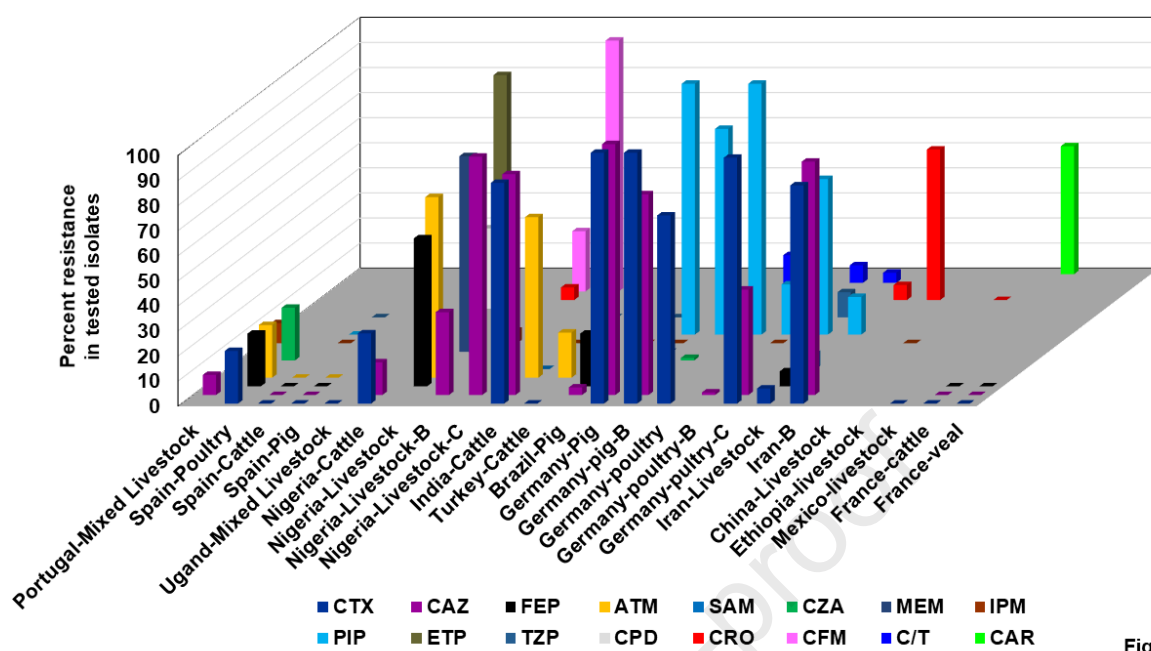


Figure 7

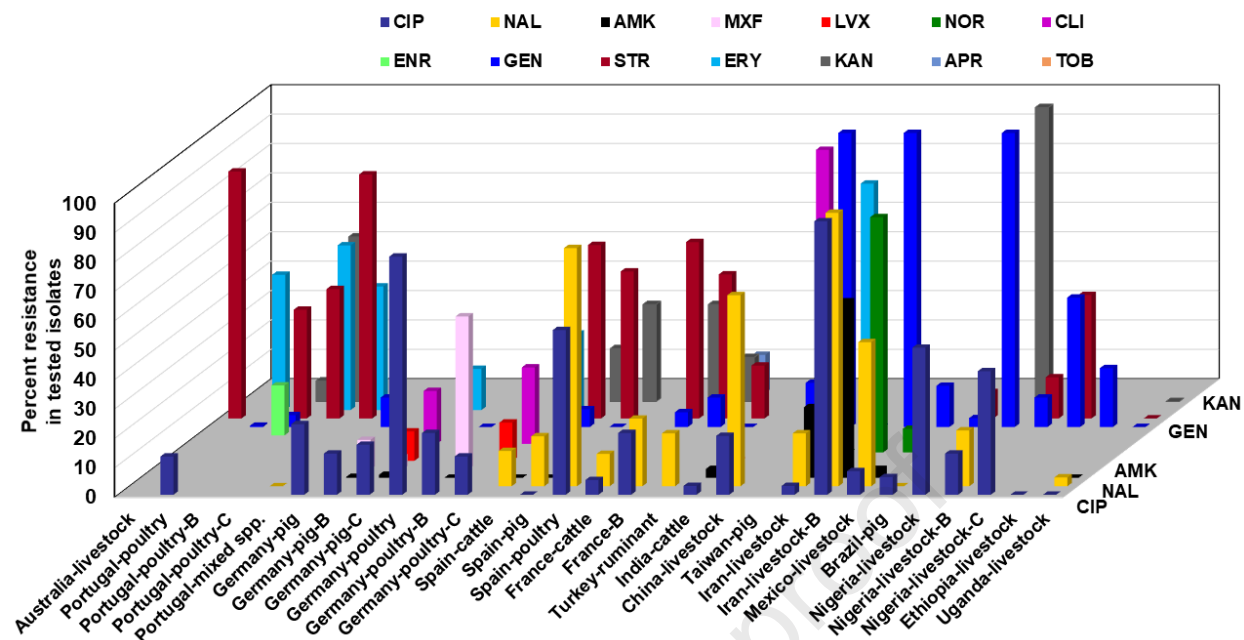


Figure 8

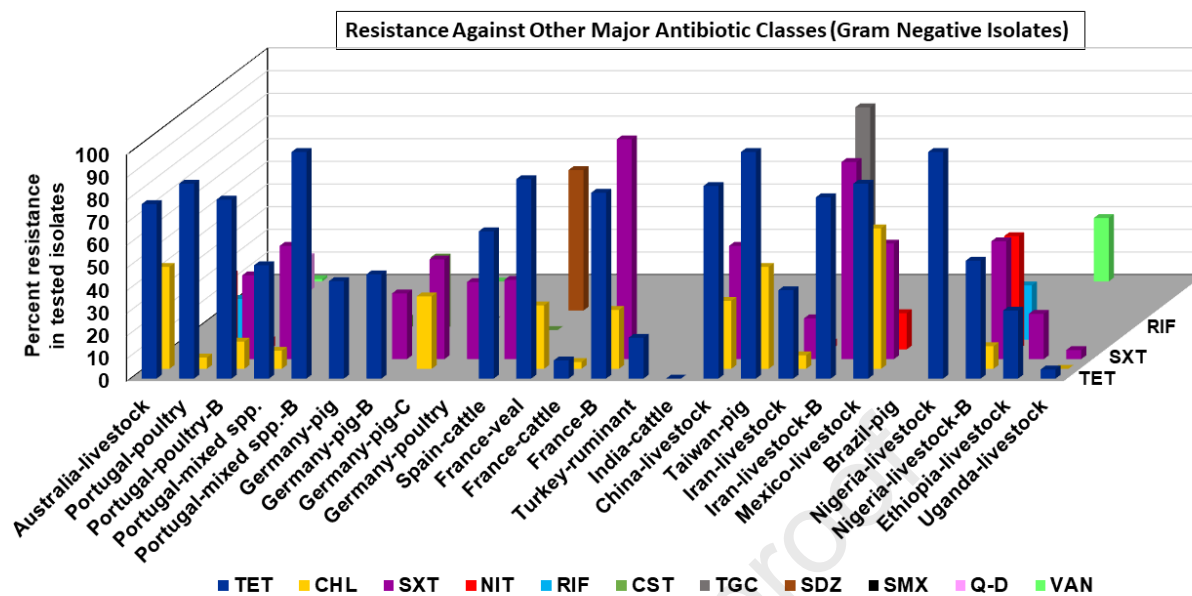


Figure 9

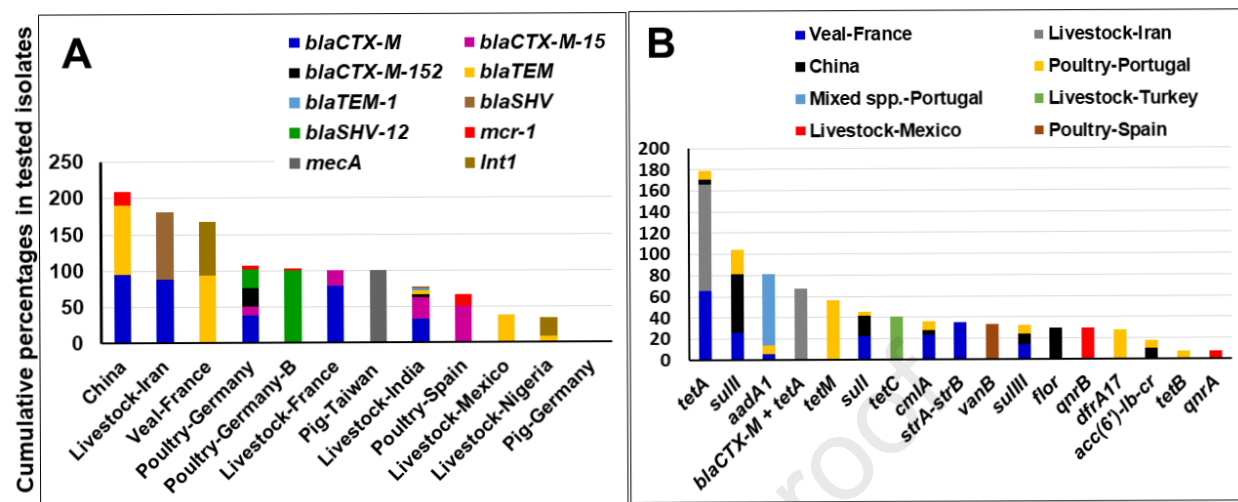


Figure 10

Highlights

- The spread of antimicrobial resistance is a recognized global One Health challenge
- Assessing abattoir effluent as environmental contaminants with antibiotic resistance
- This systematic review outlines global antibiotic resistance from abattoir effluent
- Surveillance, monitoring and decontamination of abattoir effluent is recommended

Author statement

Leo Foyle: Conceptualisation and study design, data curation, formal formal analysis and editing, supervision. Matthew Burnett, Abbey Creaser, Rachel Hens, Julia Keough, Lauren Madin, Ruby Price, Hayley Smith and Samuel Stone: All these authors contributed equally to data acquisition, data curation, preliminary data analyses and crafting of the initial draft. Robert Kinobe: Conceptualisation and study design, data acquisition, data curation, formal data analysis, writing and editing, supervision.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: